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Article *in* Microbial Pathogenesis · December 2017

DOI: 10.1016/j.micpath.2017.12.040

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Human endogenous retrovirus *env* genes: Potential blood biomarkers in lung cancer

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ARTICLE INFO

Keywords:
Lung cancer
HERV
Biomarker
Diagnosis
Blood

ABSTRACT

Lung cancer, the leading cause of cancer mortality, needs urgent development of newly qualified diagnostic and therapeutic biomarkers. Recently, Human Endogenous Retroviruses (HERVs) have been introduced for cancer diagnosis. In this case-control study, we have collected blood samples from 60 lung cancer patients and 20 healthy controls. Quantitative gene expression analysis of various HERV *env* genes, including HERV-R, HERV-H, HERV-K, and HERV-P was performed by real-time PCR. Results indicate that expression of all four HERV *env* mRNAs is significantly increased in the blood of lung cancer patients than healthy controls (*P-values* < *0.01*). Furthermore, we have observed a positive and significant pairwise correlation between the expressions of four HERV *env* genes. The level of HERV *env* transcript in the blood of adenocarcinoma patients was generally much higher than squamous cell carcinoma (SCC) and small-cell lung cancer (SCLC) patients. Also, the expression of three HERV P, HERV H, and HERV K in the blood of lung cancer patients could significantly differentiate between adenocarcinoma and other types of lung cancer. In conclusion, these four HERV families could be considered as promising non-invasive blood-based biomarkers for prognosis, early detection, and monitoring of lung cancer.

1. Introduction

Lung cancer, the most challenging cancer type, is the second leading cause of cancer death among women, worldwide [1]. A high incidence (34.2 per 100000 for men and 13.6 per 100000 for women) and also increasing the mortality rate of the disease (from 1.18 million in 2002 to 1.59 million in 2012; 30.0 per 100000 for men and 11.1 per 100 000 for women) has put it in the spotlight of cancer researchers [2].

Of importance, the survival rate of lung cancer is strongly related to the stage at which the diagnosis is made. Lung cancer can generally be diagnosed at advanced stages and according to the reports in the United States, the 5-year relative survival rate of the disease is 54% for the localized stage, 26% for the regional stage, and 4% for the distant stage of the disease [1]. Therefore, the early and precise diagnosis of lung cancer can efficiently reduce the mortality rate. Traditionally, lung cancer is divided into Non-Small Cell Lung Cancer (NSCLC, ~85%), and Small Cell Lung Cancer (SCLC). NSCLC is further subdivided into three groups, including adenocarcinoma (~50%), squamous cell carcinoma (~35%), and large cell carcinoma (~15%) [3]. Currently, diagnosis of lung cancer is performed through the imaging technologies and pathological examinations, both of which have limitations for the comprehensive description of the disease [4,5]. Evaluating EGFR (Epidermal Growth Factor Receptor) and ALK (anaplastic lymphoma kinase) mutations are most commonly used molecular tests primarily used to help in and monitoring of the treatment process. However, these two tests can only cover a low percent of patients with NSCLC, especially adenocarcinoma, and as a result, lack of a suitable molecular biomarker is well evident in other classes of lung cancer [3]. In addition, the lack of biomarkers for diagnosis, prognosis, and prediction of lung cancer is of critical concern and urges developing newly qualified

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https://doi.org/10.1016/j.micpath.2017.12.040
Received 17 October 2017; Received in revised form 11 December 2017; Accepted 13 December 2017
Available online 20 December 2017
0882-4010/

Table 1
Primers of HERVs *env* genes for Real-Time PCR analysis.

Target Gene	Forward Primer	Reverse Primer	GenBank Accession No.
HERV-R env	5′-CATGGGAAGCAAGGGAACT-3′	5′-CTTTCCCCAGCGAGCAATAC-3′	AC073210 from Chr.7q11.21
HERV-H env	5′-TTCACTCCATCCTTGGCTAT-3′	5′-CGTCGAGTATCTACGAGCAAT-3′	AJ289711 from Chr.2q24.3
HERV-K env	5′-CACAACTAAAGAAGCTGACG-3′	5′-CATAGGCCCAGTTGGTATAG-3′	AC074261 from Chr12q14.1
HERV-P env	5′-CAAGATTGGGTCCCCTCAC-3′	5′-CCTATGGGGTCTTTCCCTC-3′	DQ247958 from Chr.14q32.12

lung cancer diagnostic and therapeutic biomarkers.

Human endogenous retroviruses (HERVs) are novel biomarkers that have been recently introduced for cancer diagnostic purposes [6–9]. These retro transposable elements have been originated from exogenous retroviruses that infected human germ line cells million years ago. Although HERVs are partial sequences that are not able to produce viral particles, they have evolutionary imposed considerable changes in the host genome [10,11]. Despite many rearrangements and mutations, some HERVs can express retroviral genes, including the envelope gene (*env*) [12]. Reactivation and overexpression of HERVs have been reported in several different cancers [13–16] such as lung cancer cell line (A549) and lung tumor tissues in comparison to normal adjacent tissues [17]. However, no comparative gene expression analysis of HERVs has been studied in the blood of lung cancer patients. Herein, in order to study biomarker capability of HERVs, the expression of *env* genes from HERV R, P, H, and K was examined in the blood samples of lung cancer patients and healthy individuals.

2. Materials and methods

2.1. Study design and participants

Whole blood samples were collected in tubes containing dipotassium EDTA anticoagulant from 60 lung cancer patients (66% male, 59.65 ± 11.25 years), including 38 (63.3%) adenocarcinomas, 10 (16.6%) squamous cell carcinoma (SCC), 12 (21.1%) small cell lung carcinoma (SCLC). Also, 20 age- and sex-matched (60% male, 49 ± 14.40 years) individuals without any history of malignancy were selected as healthy controls. Samples were immediately aliquoted and stored at −80 °C. The process of patient selection and sample preparation was performed under the supervision of the institutional review board of the Baqiyatallah Medical University, and the National Institute of tuberculosis and lung diseases Masih Daneshvari Hospital. Ethics Committee of the Baqiyatallah University of Medical Sciences approved the study protocol based on the principles expressed in the Declaration of Helsinki. After written informed consent of the participants, medical history, pathological diagnosis, and treatment data were collected by oncologists from each individual. Demographic and basic information of the participants is presented in Table 2.

2.2. DNA extraction

DNA extraction was done from whole blood samples by YTA Genomic DNA Extraction Mini Kit, according to the manufacturer's instructions (Yekta Tajhiz Azma, Iran). The quality of the extracts was determined using Nanodrop ND-1000 (Thermo Scientific Fisher, USA) and gel electrophoresis. Genomic DNA was used as positive control in PCR reactions.

2.3. RNA preparation and cDNA synthesis

Total RNA was extracted using Hybrid-R ™ Blood RNA (GeneAll, Seoul, Korea) according to the manufacturer's instruction and the quality and purity of the extracted RNA were checked by spectrophotometer (NanoDrop ND-1000). Extracted RNAs were free of genomic DNA contaminant. PCR amplification was performed pre-cDNA synthesis for confirmation. Finally, total RNA was reverse transcribed to cDNA using the cDNA synthesis kit (Thermo Scientific Fisher, USA).

2.4. Real-time PCR

Standard quantitative real-time PCR was carried out by Corbett RotorGene 6000 using SYBR Green method with RealQ PCR 2X Master Mix without ROX dye (Ampliqon A/S, Denmark). The primers of HERV *env* genes were obtained from a previous study done by Ahn and Kim [17]. These sequences were checked by Primer Blast, Oligocalc, and Gene Runner program (primer sequences are listed in Table 1). The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) housekeeping gene was included as the endogenous normalization control and amplified using the following sequences: GAPDH-1: 5′CTCTCTGCTCCTC CTGTTCG3′ and GAPDH-2: 5′ACGACCAAATCCGTTGACTC3' (GenBank accession No. NM_002046.5). PCR efficiency was estimated using LinReg PCR software [18]. All samples were run in duplicate and related to the expression level of GAPDH. Real-time PCR amplification for HERVs *env* and housekeeping genes were conducted in the hot start for 15 min at 95 °C, followed by 40 cycles of 95 °C for the 20s, 58 °C for 30 s, and 72 °C for 30 s. Real-time PCR reactions were coupled with melting curve analysis to confirm the amplification specificity. According to the reference [19], PCR dependent reference gene GAPDH was used for normalization. However, we tried to use a same amount of sample, RNA, and cDNA. Non-template controls were included for each primer pair to check for any significant level of contaminants.

2.5. Statistical methods

Statistical analyses were carried out by SPSS version 21.0 (SPSS Inc, IL, USA) and GraphPad Prism version 5 (La Jolla, CA, USA). The comparison between groups with normal distribution was conducted with one-way ANOVA and independent *t*-test. Mann–Whitney *U* test and Kruskal-Wallis H test were used for groups with non-normal distributions. The correlation analysis was performed by Spearman's Rank-Order. In order to assess the association between two categorical variables, Chi-Square or Fisher's exact test was used. $P \leq .05$ was considered statistically significant.

3. Results

3.1. Study population

Sixty-six percent of the patients and 60% of healthy controls were male ($P = .33$). The rate of smokers in the patients and healthy controls were 55.3% and 30%, respectively ($P = .01$). The mean ± SD for the age of lung cancer patients and healthy controls were 59.65 ± 11.25 and 49 ± 14.40, respectively ($P = .28$). None of the demographic characteristics were statistically significant between the two groups, except for the smoking status (Table 2).

3.2. HERV expression analysis

The *env* mRNA of HERV R, P, K, and H were compared in the blood of lung cancer patients and healthy controls. Comparative gene

Table 2
Study's characteristics between the patients and healthy controls.

Characteristics	Categories	Cases (n = 60)	Controls (n = 20)	*P*
Age (Year)[a]	–	59.65 ± 11.25	49 ± 14.40	.28
Gender[b]	Male Female	40 (66%) 20 (34%)	12 (60%) 8 (40%)	.33
Smoking status[b]	Smoker Non-smoker	33 (55.3%) 27 (44.7%)	6 (30%) 14 (70%)	**.01**
Type of Cancer[b]	Adenocarcinoma SCC SCLC	38 (63.3%) 10 (16.6%) 12 (20.1%)	– – –	–
Disease stage[b]	I (Limited) II III IV V (Extensive)	8 (13.3%) 3 (5%) 8 (13.3%) 34 (55.1%) 8 (13.3%)	– – – – –	–

[a] Mean ± SD for quantitative variables.
[b] Number (Relative Frequency %) for the categorical variable. Bold P-values indicated statistically significant at the level of 0.05.

Table 3
Differential Expressions of HERV-R, HERV-P, HERV-H, and HERV-K genes between the patients and healthy controls.

Gene	Cancer samples	Control samples	Fold change ($2^{-\Delta\Delta Ct}$)	*P value*
Herv-R	22.88 ± 8.10	5.06 ± 1.34	4.52	< .001
Herv-P	6.46 ± 2.95	1.08 ± 1.25	5.98	< .001
Herv-H	7.59 ± 3.86	1.71 ± 0.70	4.44	.005
Herv-K	2.01 ± 1.10	0.19 ± 0.08	10.57	.001

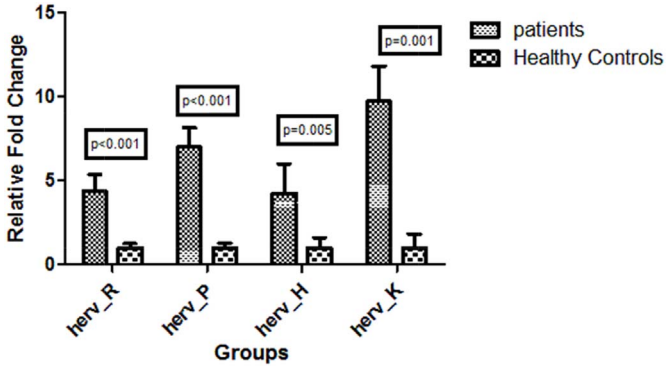

Fig. 1. Relative fold changes of HERV genes among the patients (n = 60) and healthy controls (n = 20).

expression analysis indicated an increased expression of *env* in the blood of lung cancer patients (Table 3). The highest fold change of expression was dedicated to the HERV K (fold change = 10.57, $P = .001$), followed by HERV P (fold change = 5.98, $P < .001$), HERV R (fold change = 4.52, $P < .001$), and HERV H (fold change = 4.44, $P = .005$), respectively. Expression data for all four HERVs in the patient and control groups are summarized in Table 3. Diagram of relative fold change is represented in Fig. 1.

3.3. Correlation of gene expression

The expression level of all four genes showed significant positive correlation as overexpression of each gene correlates with the up-regulation of other genes (Table 4).

The association between the gene expression with age, gender, smoking status, type of cancer, and the stage of the disease is described in Table 5. HERV R has a significant positive association with age ($P = .031$). Expression levels of HERV R in females were higher than males, but this difference was not significant ($P = .176$). In addition, in the case of HERV P, we observed the very little difference between the males and females, which was not significant ($P = .95$), too. In contrast, in the cases of HERV H and HERV K, the expression level was significant as being nearly two-fold in females higher than the males (HERV H, $P = .04$, and HERV K $P = .03$). Study of gene expression data between the smoker and non-smoker patients showed an overexpression of HERV *env* genes in the smoker than non-smoker groups. In addition, the smoker group had high expression levels of HERV P and HERV H ($P = .01$ and $P = .018$, respectively). Data analysis of the type of lung cancer showed that the level of HERV *env* transcript in the blood of adenocarcinoma patients was generally much higher than SCC and SCLC patients. Additionally, the rate of HERV P, HERV H, and HERV K transcripts in the blood of lung cancer patients could distinguish significantly between three types of lung cancer. In addition, despite high differences between three types of lung cancer, the differential expression of HERV R wasn't significant. Finally, except for HERV H, the level of HERV *env* mRNA was not significantly different in the blood of patients in different disease stages. More details are shown in Table 5.

Table 4
Correlation matrix of gene expression levels of different HERVs.

	Herv-R	Herv-P	Herv-H	Herv-K
Herv-R	1	0.78, (< 0.001)[a]	0.508, (< 0.001)[a]	0.75, (< 0.001)[a]
Herv-P	–	1	0.54, (< 0.001)[a]	0.821, (< 0.001)[a]
Herv-H	–	–	1	0.757, (< 0.001)[a]
Herv-K	–	–	–	1

(Data in each cell shown as the Spearman's rank correlation coefficient, (*P*), [a] significant correlation at 0.05 level).

4. Discussion

Reactivation of HERV genes is a promising diagnostic and therapeutic area in cancer studies. Although these elements were assessed and introduced as potential diagnostic biomarkers in several types of cancer [6], yet there are no serious and extensive studies to evaluate lung cancer diagnostic capabilities of HERV genes. Preliminary studies on lung cancer cell lines and some tumor tissues have demonstrated the overexpression of several HERV elements in lung cancer [17]. In addition, the invasive process of clinical sampling, low quantity of tissue samples as well as their inaccessibility during the course of treatment has severely restricted therapeutic applications of current lung cancer biomarkers [20–22]. We studied the differential expressions of HERV *env* genes (K, P, R, and H) in blood samples of three different lung cancer patients (adenocarcinoma, squamous cell carcinoma, small cell lung carcinoma). Among these groups, HERV K is one of the most studied HERVs in cancers, including breast cancer [6], ovarian cancer [23], and prostate cancer [24], and has been a candidate as a diagnostic and prognostic biomarker. According to our knowledge, there is not any report about the HERV K overexpression in the blood sample of lung cancer patients, to date. The present study has shown for the first time a significant overexpression of HERV K *env* in blood samples of lung cancer patients. According to our results, more differentiation in the expression of HERV K may nominate it as the best candidate biomarker among other HERV elements. It is shown that HERV-R is expressed in most human tissues at different levels and its *env* sequences are conserved in primates [25]. On the other hand, overexpression of HERV R was reported in several cancers, including lung cancer [26]. The significant increase in the HERV R *env* gene expression found here supports previous findings.

HERV H, the largest family of HERVs with more than 1000 copies in the human genome [27], was another group of HERV elements

Table 5
Association between age, gender, smoking status, stage of the disease, and types of cancer with gene expressions in the patients.

Characteristics	Categories	Herv-R	Herv-P	Herv-H	Herv-K
Age (Year)	–	0.311	0.162	0.197	0.09
	P value	**0.031**	0.27	0.26	0.69
Gender	Male	28.94 ± 8.45	7.07 ± 1.53	7.19 ± 2.50	1.57 ± 0.34
	Female	17.51 ± 4.84	6.93 ± 2.03	12.56 ± 5.47	2.95 ± 1.04
	P value	0.176	0.95	**0.04**	**0.03**
Smoking status	Smoker	34.32 ± 10.71	8.84 ± 2.07	12 ± 3.79	2.22 ± 0.62
	Non-smoker	13.15 ± 2.62	4.81 ± 1.20	6.87 ± 3.67	1.81 ± 0.72
	P value	0.065	**0.01**	**0.018**	0.069
Type of cancer	Adenocarcinoma	33.40 ± 9.39	8.40 ± 1.75	12.32 ± 3.69	2.98 ± 0.72
	SCC	13.79 ± 2.85	4.31 ± 1.81	1.63 ± 0.41	0.97 ± 0.27
	SCLC	11.97 ± 2.68	3.37 ± 1.01	2.15 ± 0.79	1.21 ± 0.88
	P value	0.282	**0.001**	< **0.001**	**0.02**
Disease stage	I (Limited)	15.43 ± 4.16	3.55 ± 4.16	–	–
	II	23.13 ± 11.53	4.32 ± 1.59	–	–
	III	11.53 ± 5.18	5.16 ± 3.77	–	1.38 ± 1.02
	IV	12.76 ± 2.01	4.36 ± 1.37	1.57 ± 0.19	0.66 ± 0.14
	V (Extensive)	12.74 ± 3.89	6.76 ± 1.86	1.79 ± 0.14	0.49 ± 0.23
	P value	0.66	0.96	**0.045**	0.13

Bold P-values indicates statistical significance at the level of 0.05.

investigated in this study. In comparison with other HERVs, HERV H has less evolutionary changes indicating its different dynamics. Based on a previous research, HERV H is necessary for the appropriate function of stem cells [28]. It was shown that HERV H has a basal expression in the normal lung, but it is increased in lung cancer cell line (A549) [29] as well as the lung tumor [17]. Following previous studies, we examined expression level changes of HERV H in the blood samples of lung cancer patients and demonstrated significant differences in *env* mRNA levels of HERV H in comparison with normal individuals.

HERV-P was the fourth family of HERVs evaluated in this study. Expression analysis of HERV-P in cancer cell lines suggested that this family of retroelements may have a potential role in carcinogenesis [30]. This HERV family has 20 to 40 copies per haploid genome [31]. Herein, overexpression of HERV-P *env* gene in lung cancer patients showed a significant difference with normal cases. On the other hand, it seems that the direct and significant pairwise correlation between four HERV *env* genes expression may implicate a similar reactivation process during cancer development. In other words, cancerous conditions may have similar effects on the reactivation pathways of these HERVs. Previously, Rhyo et al. had reported a similar regulatory pattern for these four HERVs in the blood sample of breast cancer patients [7]. Generally, our results are consistent with the findings of Rhyo et al. in breast cancer. Among four studied HERVs families, HERV K *env* was the most differentially expressed gene with very low or even no expression in the normal subjects compared to the significant increase in lung cancer patients. Classification of cancer patients is a priority in personalized medicine that has created a great promise for effective management. Molecular biomarkers play a central role in this model of medicine, and therefore, the ability of HERVs to distinguish among three types of lung cancer is a promising issue. Except for HERV R, differential expression of HERVs was statistically significant in three types of lung cancer. In general, expression levels of HERVs can be considered as criteria to differentiate adenocarcinoma from other types of lung cancer. Of note, more comprehensive and concise experiments are required to prove the claim.

Cigarette smoking is considered as the major risk factor for lung cancer in all stages of diagnosis and treatment [32]. Commercial tobacco smoke contains more than 5000 chemicals, mostly known as carcinogens [33]. The association between HERVs overexpression and smoking was already demonstrated in the normal urothelium and in a newly established *in vitro* cell line model [34]. Also, our results showed that the smoking status is associated with the increase expression of HERVs. On the other hand, it was recently indicated that HERV-K *env* plays important roles in the tumorigenesis and metastasis of breast cancer [14]. The smoking possibly increases the risk of lung cancer via the overexpression of HERV elements. It is recommended to investigate molecular pathways involved in the carcinogenesis of smoking. Interestingly, expression of HERVs was increased with the disease progression. Despite differences observed in the expression of HERVs among different stages, only HERV H was significantly increased. The majority of clinical samples used in this study belonged to the stage IV of the disease. It seems that collecting sufficient samples from different stages may result in significant increases in other HERVs.

In conclusion, these four HERV families may be considered as promising biomarker candidates for prognosis, early detection, and monitoring of lung cancer. In many cases, significant differences in HERVs expression between lung cancer patients and normal individuals can reduce the false positive and false negative cases of imaging techniques. More investigations are needed to assess the association between the HERVs expression levels and progression, early detection and therapeutic efficacy for the disease. Highlighted capabilities of these retroelements in the management of lung cancer may accelerate the personalized therapy of lung cancer.

Conflicts of interest

Authors claim no conflict of interests.

Acknowledgment

The authors would like to thank Baqiyatallah University of Medical Sciences and the National Institute of Tuberculosis and Lung Diseases of Masih Daneshvari Hospital for their support and assistance. It should be noted that this paper is extracted from PhD thesis of Mokhtar Zare.

References

[1] L.A. Torre, R.L. Siegel, A. Jemal, Lung Cancer Statistics. Lung Cancer and Personalized Medicine: Springer, (2016), pp. 1–19.
[2] T.-Y.D. Cheng, S.M. Cramb, P.D. Baade, D.R. Youlden, C. Nwogu, M.E. Reid, The international epidemiology of lung cancer: latest trends, disparities, and tumor characteristics, J. Thorac. Oncol. 11 (2016) 1653–1671.
[3] P.T. Cagle, T.C. Allen, R.J. Olsen, Lung cancer biomarkers: present status and future developments, Arch. Pathol. Lab Med. 137 (2013) 1191–1198.
[4] E.F. Patz, P. Pinsky, C. Gatsonis, J.D. Sicks, B.S. Kramer, M.C. Tammemägi, et al., Overdiagnosis in low-dose computed tomography screening for lung cancer, JAMA intern. med. 174 (2014) 269–274.
[5] K.E. Konopka, Diagnostic Pathology of Lung Cancer. Seminars in Respiratory and Critical Care Medicine, Thieme Medical Publishers, 2016, pp. 681–688.
[6] F. Wang-Johanning, M. Li, F.J. Esteva, K.R. Hess, B. Yin, K. Rycaj, et al., Human endogenous retrovirus type K antibodies and mRNA as serum biomarkers of early-

stage breast cancer, Int. J. Canc. 134 (2014) 587–595.
[7] D.W. Rhyu, Y.J. Kang, M.S. Ock, J.W. Eo, Y.H. Choi, W.J. Kim, et al., Expression of human endogenous retrovirus env genes in the blood of breast cancer patients, Int. J. Mol. Sci. 15 (2014) 9173–9183.
[8] K. Iramaneerat, P. Rattanatunyong, N. Khemapech, S. Triratanachat, A. Mutirangura, HERV-K hypomethylation in ovarian clear cell carcinoma is associated with a poor prognosis and platinum resistance, Int. J. Gynecol. Canc. 21 (2011) 51–57.
[9] N.B. Seniuta, A.M. Kleiman, A.A. Triakin, A.I. Karseladze, V.M. Shelepova, S.A. Tiuliandin, et al., Antibodies to stuctural proteins of endogenous retrovirus of the HERV-K/HTDV family as markers of human germ cell tumors, Vopr. Virusol. 51 (2006) 17–21.
[10] N. Bannert, R. Kurth, The evolutionary dynamics of human endogenous retroviral families, Annu. Rev. Genom. Hum. Genet. 7 (2006) 149–173.
[11] V. Blikstad, F. Benachenhou, G.O. Sperber, J. Blomberg, Evolution of human endogenous retroviral sequences: a conceptual account, Cell. Mol. Life Sci. 65 (2008) 3348–3365.
[12] N. de Parseval, V. Lazar, J.-F. Casella, L. Benit, T. Heidmann, Survey of human genes of retroviral origin: identification and transcriptome of the genes with coding capacity for complete envelope proteins, J. Virol. 77 (2003) 10414–10422.
[13] G. Kassiotis, Endogenous retroviruses and the development of cancer, J. Immunol. 192 (2014) 1343–1349.
[14] F. Zhou, M. Li, Y. Wei, K. Lin, Y. Lu, J. Shen, et al., Activation of HERV-K Env protein is essential for tumorigenesis and metastasis of breast cancer cells, Oncotarget 7 (51) (2016) 84093.
[15] C.S. Mullins, M. Linnebacher, Human endogenous retroviruses and cancer: causality and therapeutic possibilities, World J. Gastroenterol. 18 (2012) 6027–6035.
[16] R.F. Downey, F.J. Sullivan, F. Wang-Johanning, S. Ambs, F.J. Giles, S.A. Glynn, Human endogenous retrovirus K and cancer: innocent bystander or tumorigenic accomplice? Int. J. Canc. 137 (2015) 1249–1257.
[17] K. Ahn, H.S. Kim, Structural and quantitative expression analyses of HERV gene family in human tissues, Mol. Cell. 28 (2009) 99–103.
[18] D. Robledo, J. Hernández-Urcera, R.M. Cal, B.G. Pardo, L. Sánchez, P. Martínez, et al., Analysis of qPCR reference gene stability determination methods and a practical approach for efficiency calculation on a turbot (Scophthalmus maximus) gonad dataset, BMC Genom. 15 (2014) 648.
[19] M.T. Dorak, Real-time PCR, Taylor & Francis, 2007.
[20] H.X. Zhou, M.X. Yang, Y. Wang, W.M. Cao, K.F. Lu, L.Y. Zong, et al., Plasma LUNX mRNA, a non-invasive specific biomarker for diagnosis and prognostic prediction of non-small cell lung cancer, Am J Cancer Res. 6 (2016) 452–458.
[21] K. O'Shea, S.J. Cameron, K.E. Lewis, C. Lu, L.A. Mur, Metabolomic-based biomarker discovery for non-invasive lung cancer screening: a case study, Biochim. Biophys. Acta 1860 (2016) 2682–2687.
[22] H. Dou, Y. Wang, G. Su, S. Zhao, Decreased plasma let-7c and miR-152 as non-invasive biomarker for non-small-cell lung cancer, Int. J. Clin. Exp. Med. 8 (2015) 9291–9298.
[23] F. Wang-Johanning, J. Liu, K. Rycaj, M. Huang, K. Tsai, D.G. Rosen, et al., Expression of multiple human endogenous retrovirus surface envelope proteins in ovarian cancer, Int. J. Canc. 120 (2007) 81–90.
[24] L. Agoni, C. Guha, J. Lenz, Detection of human endogenous retrovirus K (HERV-K) transcripts in human prostate cancer cell lines, Front. oncol. 3 (2013) 180.
[25] H.S. Kim, J.M. Yi, H. Hirai, J.W. Huh, M.S. Jeong, S.B. Jang, et al., Human Endogenous Retrovirus (HERV)-R family in primates: chromosomal location, gene expression, and evolution, Gene 370 (2006) 34–42.
[26] Y.-J. Kang, J.-O. Jo, M.S. Ock, H.-K. Chang, K.-W. Baek, J.-R. Lee, et al., Human ERV3-1 env protein expression in various human tissues and tumours, J. Clin. Pathol. 67 (2014) 86–90.
[27] J.A. Levy, The Retroviridae, Springer Science & Business Media, 2013.
[28] P. Gemmell, J. Hein, A. Katzourakis, Phylogenetic analysis reveals that ERVs "die young" but HERV-H is unusually conserved, PLoS Comput. Biol. 12 (2016) e1004964.
[29] J.M. Yi, H.M. Kim, H.S. Kim, Human endogenous retrovirus HERV-H family in human tissues and cancer cells: expression, identification, and phylogeny, Canc. Lett. 231 (2006) 228–239.
[30] J.M. Yi, K. Schuebel, H.S. Kim, Molecular genetic analyses of human endogenous retroviral elements belonging to the HERV-P family in primates, human tissues, and cancer cells, Genomics 89 (2007) 1–9.
[31] H.B. Urnovitz, W.H. Murphy, Human endogenous retroviruses: nature, occurrence, and clinical implications in human disease, Clin. Microbiol. Rev. 9 (1996) 72–99.
[32] A. Xie, B. Croce, D.H. Tian, Smoking and lung cancer, Ann. Cardiothorac. Surg. 3 (2014) 221.
[33] R. Talhout, T. Schulz, E. Florek, J. Van Benthem, P. Wester, A. Opperhuizen, Hazardous compounds in tobacco smoke, Int. J. Environ. Res. Publ. Health 8 (2011) 613–628.
[34] U. Gabriel, A. Steidler, L. Trojan, M.S. Michel, W. Seifarth, A. Fabarius, Smoking increases transcription of human endogenous retroviruses in a newly established in vitro cell model and in normal urothelium, AIDS Res. Hum. Retrovir. 26 (2010) 883–888.